Studies on Genetic and Hormonal Sex Control in Domesticated Rainbow Trout. II. Use of Methyltestosterone for Masculinization and Sterilization in Cultured Rainbow Trout *(Salmo gairdneri* Richardson*)*

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STUDIES ON GENETIC AND HORMONAL SEX CONTROL IN DOMESTICATED RAINBOW TROUT. II. USE OF METHYLTESTOSTERONE FOR MASCULINIZATION AND STERILIZATION IN CULTURED RAINBOW TROUT (*Salmo gairdneri* Richardson)

by

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ABSTRACT

Solar, I. I. and E. M. Donaldson. 1985. Studies on genetic and hormonal sex control in a domesticated rainbow trout. II. Use of methyltestosterone for masculinization and sterilization cultured rainbow trout (Salmo gairdneri Richardson). Can. Tech. Rep. Fish. Aquat. Sci. 1380: 9 p.

The oral administration of 17α-methyltestosterone to a domestic strain of rainbow trout (Spring Valley) from first feeding for periods of 60, 90, and 120 days at concentrations ranging from 1 to 100 mg/kg diet produced results similar to those observed in wild rainbow trout (Pennask Lake) after the same treatment. One hundred percent males were produced with 1 and 3 mg/kg fed for 90 and 120 days. A high proportion of these males had altered testes with germinal elements among areas of sterile tissue. Up to 87% sterile fish were produced with 50 mg/kg for 60 days. High doses of the androgen also induced feminization of the gonads. In the group receiving 100 mg/kg for 120 days 34% of the gonads were feminized. The aromatization of androgens to estrogens is discussed as a possible mechanism for the paradoxical feminizing effect of the androgen treatment.

Key words: androgen treatment, sex reversal, sterility, methyltestosterone, "paradoxical", feminization, steroids, Salmo gairdneri

RESUME

Solar, I. I. and E. M. Donaldson. 1985. Studies on genetic and hormonal sex control in a domesticated rainbow trout. II. Use of methyltestosterone for masculinization and sterilization cultured rainbow trout (Salmo gairdneri Richardson). Can. Tech. Rep. Fish. Aquat. Sci. 1380: 9 p.

L'administration par voie orale de 17a-méthyltestostérone à une souche de truites arc-en-ciel d'élevage (Spring Valley) à partir du moment où l'on a commencé à les alimenter, pendant des périodes de 60, 90 et 120 jours, à des concentrations variant de 1 à 100 mg/kg de nourriture, a produit des résultats similaires à ceux observés chez des truites arc-en-ciel sauvages (Pennask Lake) après qu'elles aient subi le même traitement. En donnant 1 et 3 mg/kg de ce produit pendant 90 et 120 jours 100% de mâles ont été produits. Une grande partie de ces mâles avaient de testicules modifés contenant des cellules germinales entre des zones de tissu stérile. Jusqu'à 87% des poissons stériles ont été produits en donnant 50 mg/kg pendant 60 jours. Des doses élevées d'androgène ont également provoqué la féminisation des gonades. Dans le groupe qui a reçu 100 mg/kg pendant 120 jours, 34% des gonades se sont féminisées. On discute de la transformation aromatique des androgènes en estrogènes comme mécanisme possible pour expliquer l'effet paradoxal de féminisation du traitement aux androgènes.

Mots-clés: tratement aux androgènes, changement de sexe, stérilité, méthyltestostérone, féminisation "paradoxale", stéroides, Salmo gairdneri

INTRODUCTION

The intensive rearing of salmonid fishes, particularly the mariculture of Atlantic salmon and rainbow trout, is a fast expanding industry in Europe. More recently there has been a growing interest in British Columbia in the culture of Pacific salmon and also rainbow trout in sea water pens.

In the case of rainbow trout mariculture, market considerations indicate that 250-300 g and 3-5 kg could be the appropiate sizes for portion and meal size trout, repectively. However, the achievement of these larger sizes is difficult and frequently expensive because of the tendency of the fish, particularly males, to mature sexually at an early age. Manipulation of the maturation process by the artificial production of all-female or sterile stocks could reduce or eliminate these problems. Several experimental approaches have been considered towards this objective (auto-immune gonadal rejection, surgical gonadectomy, genetic techniques: hybridization, gynogenesis and polyploidy, and hormonal techniques: sex reversal and sterilization; for review see Donaldson and Hunter 1982; Hunter and Donaldson 1983).

In a previous study (Solar et al., 1984) we submitted fry of wild rainbow trout (spring-summer spawner) to a range of doses of the androgen 17α-methyltestosterone to induce sterilization and masculinization (as a first step in the production of all-female stock). The present study was undertaken to further test these androgen treatments in a related wild population and to compare the effect of similar treatment in the Spring Valley strain of rainbow trout (winter spawner), a hatchery developed stock considered well suited for mariculture for its favourable domestic traits, hardiness, fast growth and easy acclimation to seawater transfer (W. C. Clarke pers. comm. 1985).

MATERIALS AND METHODS

Eggs and sperm from Spring Valley rainbow trout reared in seawater netpens at the Pacific Biological Station, Nanaimo, B.C. were transported to the laboratory and dry fertilized according to standard procedures. Swim-up fry in groups of approximately 200 were placed in 50 l tanks supplied with aerated well water at 10-11°C. Lighting was provided by overhead fluorescent fixtures timed with the natural photoperiod.

Starting at 730°C days from fertilization (370°C days from median hatching) the fry were fed Oregon moist pellets (OMP) treated with different doses of 17α-methyltestosterone (MT) prepared as described by Hunter et al. (1982). The doses and durations of the treatments were the same as in the previous experiment with the wild stock: 1, 3, 9, 25, 50 and 100 mg MT/kg diet administered for 60, 90, and 120 days (day 37 to 97, day 37 to 127, and day 37 to 157).

The histological procedures for the determination of sex and gonadal condition, and the statistical analysis (factorial analysis of variance) to examine the effect and dependency of the doses and the duration of the treatments on the sex ratios produced were also identical to those applied in our study on the wild stock of rainbow trout (Solar et al. 1984).

RESULTS

Normal 1:1 male-female ratios were found in the control groups examined at the end of each period of treatment. The experimental groups showed substantial variation from normal ratio. The proportions of sexes and gonadal conditions are shown in Figure 1. The cytological appearance of the gonads was clear and consistent in the control groups. A wide range of conditions was observed in the gonads of the treated groups which can be summarized as follows:

a) Normal and underdeveloped ovaries with few or several oocytes at the perinucleolar stage. In some cases the ovary, though small, had numerous oocytes closely resembling the normal ovary of the control females;

b) Normal testes, similar to the control testes. Advanced spermatogenesis was more frequently found in the low dose groups than in the controls;

c) Affected testis consisting of variable areas of spermatogonia and connective tissue. Blood vessels and sperm duct were usually present along with a thickened hilum and enlarged tunica;

d) Sterile testis, usually very small consisting solely of connective tissue or larger but with extense areas devoid of cytological elements, a sac-like structure with an ephithelial tunica.

Based on the preceding characteristics the fish were classified and grouped as female, male, partially sterile, and sterile.

Figure 1 shows that fish having female germinal elements were present (2-26%) in all groups treated for 60 days with the exception of the 25 mg/kg MT group. The remaining fish were males with normal testes or having altered gonads with localized cystic areas of spermatogonia (2-96%). Predominantly sterile gonads were produced with doses of 25, 50, and 100 mg/kg (77, 87, and 72%, respectively).

Treatments lasting for 90 and 120 days produced similar results. The highest proportions of males (100%) were observed in the groups treated with 1 mg/kg for 90 days and 3 mg/kg for 120 days. However, about 1/4 of these males had affected testes with reduced areas of spermatids and proliferation of sterile tissue. The frequency of gonads showing female germinal elements, in some cases amounting to almost a normal ovary, increased with the dose to a high of 34.5% in the group which received 100 mg/kg for 120 days.

Two way analysis of variance (factorial ANOVA) demonstrated, as is apparent by observation of Figure 1, that the sex ratios and gonadal conditions observed are significantly dependent on dosage with little or no variation due to duration of the treatment, without evidence of dose-duration interaction.

No dose related mortality was observed during early rearing. The growth of the treated groups was uniform and not significantly different from controls with the groups receiving 100 mg/kg showing, unexpectedly good growth and superior condition factor (Table 1).

The fish are being reared beyond the duration of the experiment and except for an outbreak of vibriosis, which was successfully controlled by medication (Sulfamerazine), they have shown good survival and growth.

DISCUSSION

The results of this study indicate that the effect of the androgen 17α-methyltestosterone (MT) is essentially similar in both domestic and wild rainbow trout. The fish were highly responsive to the exogenous steroid administration showing marked modifications in the distribution of sex types and gonadal morphology as a result of the treatments.

As in our previous study on the wild stock (Solar et al. 1984) a paradoxical dose related, increase of treated fish showing developing oocytes was observed. Comparatively, the feminization process caused by the high doses of androgen was more intense in the domestic stock than in the wild population. Some of the ovaries observed in the groups treated for 90 and 120 days were very similar to the control ovary and one replicate group receiving 100 mg/kg for 120 days included a proportion of female fish of 53%. As a consequence of the progressive increase of numbers of female fish caused by the high doses of MT, the highest percentage of sterile fish produced was 87% (50 mg/kg diet for 60 days).

The paradoxical effect of steroid administration is a relatively frequent occurrence in lower vertebrates (Table 2). Hackmann (1974) has hypothesized two possible causes for the phenomenon, as observed in cichlid fishes:

a) The exogenous androgen would cause ovarian differentiation by inhibition of the biosynthesis of the endogenous androgen;

b) The feminization would be caused by the conversion of the administered androgen into estrogen by metabolic processes (aromatization in presence of the aromatase enzyme).

Studies by Crim et al. (1981) seem to reinforce the second possibility. Crim et al. (1981) observed changes in the levels of gonadotropic hormone in sexually immature rainbow trout treated with aromatizable androgenic steroids, including 17α-methyltestosterone, while

non-aromatizable androgens were ineffective.

Some naturally occurring androgens present in the plasma of teleosts (11-ketotestosterone (KT) and 11β-hydroxytestosterone) are not aromatized to estrogens but other androgens such as testosterone (T) and androstenedione are aromatized to estradiol and estrone, respectively. Additionally, the aromatization processes of androgens into estrogens may occur not only at the level of the pituitary, brain and gonads, but also peripherally from circulating plasma androgens in bone, skin, and adipose tissue (Callard 1983).

It is concluded that the use of non-aromatizable androgens, i.e. 11-ketotestosterone or 11β-hydroxytestosterone, could be an alternative androgen treatment for the effective sterilization of rainbow trout. The relatively lower androgenic potency of KT and the high cost of the hormone would currently limit the practicability of its use at a commercial level. A different approach would be the modification of treatment regimes using MT or T, possibly including eyed egg and alevin immersions.

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Table 1. Growth data at the end of each treatment period of feeding 0-100 mg/kg diet 17α-methyltestosterone (MT), mean ± SD.

	MT dose mg/kg diet	D 97		D 127		D 157	
Length (cm ± SD)	0	5.0	0.4	6.3	0.5	7.9	0.9
	1	4.8	0.4	6.3	0.6	8.1	0.6
	3	4.9	0.5	6.3	0.5	8.0	0.7
	9	5.0	0.4	6.3	0.5	8.0	0.6
	25	4.8	0.6	6.0	0.5	7.7	0.5
	50	4.8	0.4	6.2	0.5	7.8	0.7
	100	5.0	0.4	6.3	0.6	8.3	0.7
Weight (g ± SD)	0	1.3	0.3	2.8	0.7	6.4	2.6
	1	1.4	0.4	3.1	0.8	7.0	1.7
	3	1.2	0.4	3.0	0.8	6.7	1.7
	9	1.4	0.3	3.0	0.7	6.5	1.5
	25	1.3	0.3	2.6	0.6	5.9	1.2
	50	1.3	0.3	2.9	0.6	6.3	1.6
	100	1.4	0.4	3.2	0.8	8.0	1.9
Condition factor[1] (K ± SD)	0	1.06	0.06	1.10	0.06	1.24	0.08
	1	1.12	0.11	1.18	0.08	1.27	0.06
	3	1.05	0.14	1.16	0.11	1.26	0.07
	9	1.14	0.09	1.19	0.09	1.23	0.06
	25	1.10	0.11	1.20	0.08	1.24	0.05
	50	1.12	0.10	1.22	0.14	1.29	0.09
	100	1.14	0.07	1.25	0.09	1.38	0.10

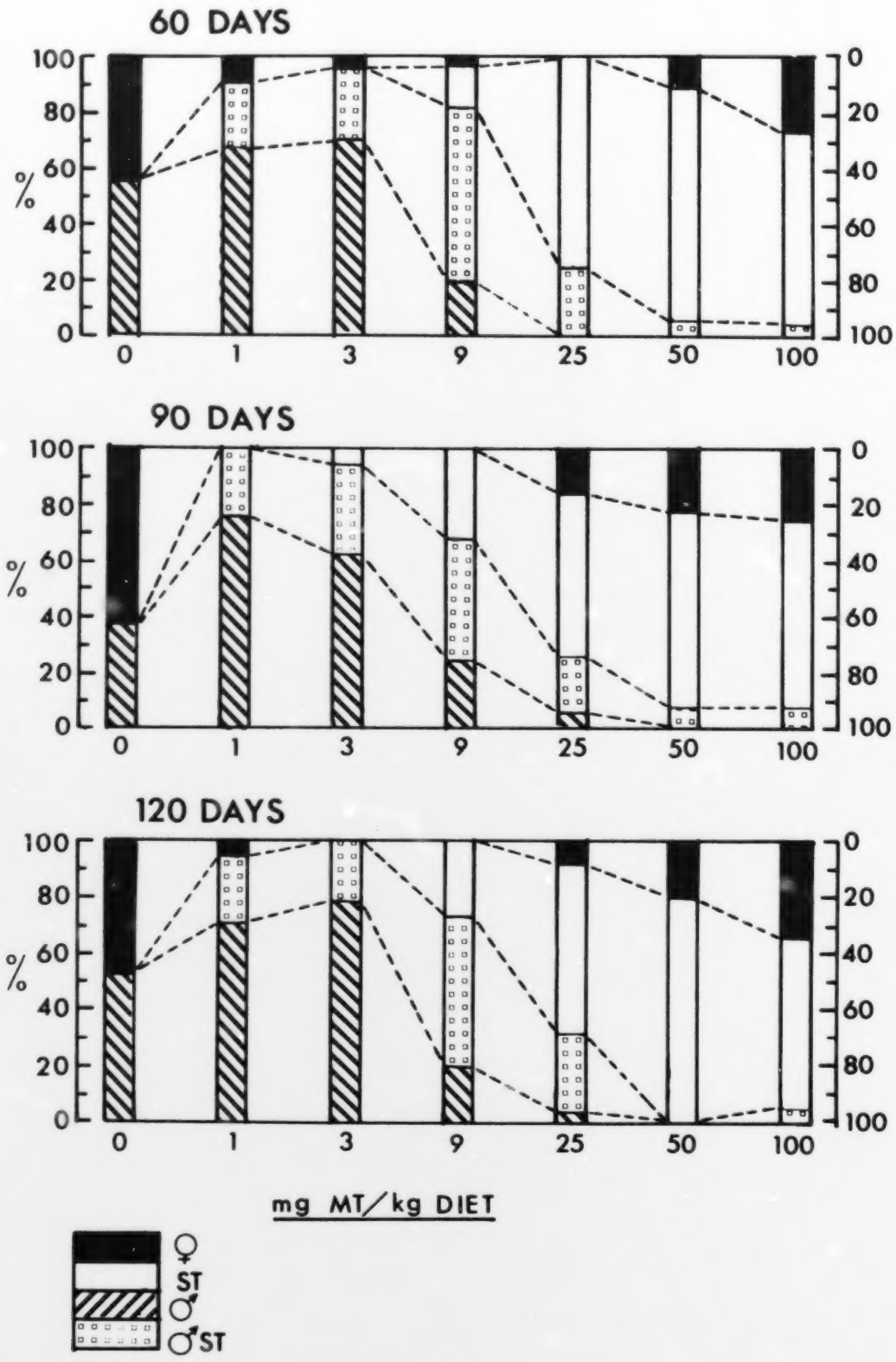
D = days post-hatching (age) at time of sampling.

[1] $K = W/L^3 \times 100$.

Table 2. Paradoxical effects of steroid administration.

Species	Steroid	Effect	Reference
Rana esculenta	Estrone 250-100 µg/l	100%	Padoa (1936)
Pleurodeles waltlii	Testosterone	100%	Gallien (1950)
Rana catesbeiana	Estradiol	Masculinization	Hsu et al. (1978)
Hemihaplochromis multicolor	Testosterone Propionate or Methyltestosterone	Feminization	Muller (1969)
Tilapia mossambica	Testosterone Propionate 500 µg/l	Strong Feminization	Hackmann (1974)
Tilapia heudeloti	Testosterone Propionate 500 µg/l	Strong Feminization	Hackmann (1974)
Cichlasoma biocellatum	Methyltestosterone	Feminization	Hackmann (1974)
Tilapia mossambica	Methyltestosterone 1000 mg/kg	Feminization	Nakamura (1975)
Poecilia reticulata	Methyltestosterone 1000 - 2000 µg/g diet	Feminization	Takahashi (1977)
Ictalurus punctatus	Ethynyltestosterone	Feminization	Goudie et al. (1983
Salmo gairdneri	Methyltestosterone	Feminization	Solar et al. (1984)

Fig. 1 - Effect of oral administration of 17α-methyltestosterone on sex differentiation (gonadal morphology) of cultured rainbow trout treated for periods of 60, 90 and 120 days. (ST = sterile, ♂ ST = partially sterile)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